REMARKS

With entry of this amendment, claims 1 - 94, and 96-143 are pending in the Application. By this amendment, claims 11, 39, 55, 131, 132, 134, 136, 139-141, and 143 have been amended for clarity in accordance with the Examiner's suggestions. All of the amendments presented herein are fully supported by the specification and no new matter has been added to the application. Entry of this amendment is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Appendix. Version with Markings to Show Changes Made."

Information Disclosure Acknowledgement

Applicants note that the Supplemental Information Disclosure Statement with PTO 1449 submitted on May 23, 2000 was received by the Office on May 26, 2000 (copy with PTO postcard acknowledgment enclosed). However, a formal copy of the 1449 checked off by the Examiner has not yet been received (although a checked-off copy of the original IDS and a later-filed Second Supplemental IDS submitted July 24, 2000 have been received by Applicants). Formal acknowledgment of the first Supplemental IDS by the Examiner is therefore earnestly solicited.

Claim Objections

Claim 55 is objected to for reasons of record. Briefly, the Office contends that it is unclear whether claim 52 is intended to encompass "providing some or all of the NPL proteins from a coinfecting PIV instead of providing them from an expression vector (or several expression vectors), or if the intent is to provide one or more of the NPL proteins in duplicate, from both an expression vector and a coinfecting PIV." Applicants respond by noting that claim 55 has been amended herein for clarity, in accordance with the Examiner's concerns, to delete the reference to supplying one or more of the N, P and L proteins "by coinfection with PIV." This amendment clearly removes the potential ambiguity noted by the

Office. At the same time, it is noted that the claims are not closed to the possible inclusion of a coinfected PIV in more detailed embodiments to supplement the claimed method (e.g., by coexpression of one or more of the N, P or L proteins from the coinfected virus).

Patentability Under 35 U.S.C. §112

Claims 48-90, 135, and 136 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. Specifically, the Office contends that the content and relationship of claim 52 and its dependent claim 55 impose ambiguity, particularly in the recitation in claim 55 that "at least one of the N, P, and L proteins is supplied by coinfection with PIV". This rejection is obviated by the amendment to claim 55, noted above, which deletes the allegedly objectionable reference to protein expression by viral coinfection.

Claims 39, 111, 131, 139 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. In particular, the Office states that "rcp45 is a recombinant version of JS cp45, and therefore has attenuating mutations in all segments of the genome. How can a chimeric virus replace a genome segment without replacing some of these mutations? How can it be simultaneously chimeric and have the full complement of attenuating mutations present in rcp45?" The first part of the Office's statement is correct, and the second portion is insightful in the sense that chimeric replacement of a "wild-type" gene in a JS cp45 background would displace any cp45 mutations present in the substituted gene or genome segment. Naturally, the cp45 mutations could be engineered within the wild-type replacement gene or genome segment according to the teachings of the specification, if desired. However, the potential ambiguity arising by the reference to "mutations present in cp45" is noted.

To remedy this potential ambiguity, the Office proposes to clarify the claims by "specifying the list of sites and nucleotides, like in claims 132+" . . . "to encompass chimeric viruses with specific nucleotides at any or all of a group of specific sites (corresponding to the sites that differ from the wild-type sequence in the reference constructs)." In accordance with this suggestion, all of the subject claims have been amended to refer to the cp45 mutations that occur outside of the introduced gene(s) HN and/or F in the subject chimeric constructs. On this basis, withdrawal of the rejection is earnestly solicited.

Claims 15, 16, 30, 31, 36, 37, 39, 65- 69, 71, 72, 78, 81, 82, 105, 106, 109-111, 113, 114,117, 127, 128, 130, 131, 133, 135, 138, 139 stand rejected under 35 U.S.C. §112, first paragraph for alleged nonenablement. In particular, the Office contends that a deposit of JS cp45 is needed to support the subject claims. Applicants note that the requested deposit has been made, which clearly obviates the rejection without acquiescence or specific consideration to the merits thereof. Certification of the deposit and its terms was forwarded to the Office in a Communication filed in the application on December 7, 2000. The specification has been amended herein to include a reference to the deposit in the detailed description. For these reasons, the stated rejection is believed to be obviated.

Claims 1-10, 33-47, 73-87, 88, 89, 94, 97-101, 107-114, 116, 121-126, 129-143 stand rejected under 35 U.S.C. §112, first paragraph for alleged nonenablement. The Office contends that the specification, "while enabling for the chimeric viruses constructed in the working examples, does not reasonably provide enablement for the full scope of chimeras claimed." Applicants respectfully traverse and request clarification of this new ground for rejection.

The Office acknowledges in the rejection that Applicants' disclosure teaches effective construction and recovery of useful chimeric vaccine strains, for example, between different human PIV (HPIV) viruses (as exemplified by HPIV1-HPIV3 chimeric constructs). However, the Office rejects Applicants' claims directed to human/non-human PIV recombinants, (e.g., HPIV having a bovine PIV (BPIV) or respiratory syncytial virus (RSV) gene), for alleged nonenablement. At the same time, the Office indicates that this subject matter, rejected under 35 U.S.C. §112, first paragraph in the present application, is anticipated by the Belshe et al. reference (see, Office Action Paper No. 17, at pp. 5 and 7). This presumes that the Belshe et al. reference is fully enabling for the presently claimed subject matter (see below), which position appears at odds with the Office's instant representations arising from the rejection under 35 U.S.C. § 112, first paragraph (particularly considering that Belshe et al. provide no working examples of a live, recombinant PIV, much less of a live, recombinant, chimeric PIV).

In presenting Belshe et al. as allegedly adverse prior art, the Office only draws a limiting threshold regarding enablement of chimeric PIV constructs at the level of a PIV-measles virus chimera. More specifically, the Office states at page 9 of the Office Action (commenting on Applicants' arguments pertaining to Belshe et al.), as follows: "Applicant argues that success of making a replication-competent chimeric virus is unpredictable; this argument is convincing as applied to claims 5, 8, 9, 45, 46, 86, and 87." Notably, all of these claims relate to chimeric PIV that incorporate a gene or gene segment (e.g., a HA or F gene or gene segment) from a measles virus.

For all other chimeric PIV subject matter in the claims apart from HPIV/HPIV constructs (which the Office appears to consider fully enabled by the specification), the record remains unclear as to the Office's position concerning enablement issues. For example, is Applicants' disclosure deemed insufficient to support human/non-human PIV chimeras?, and/or PIV/non-PIV (e.g., PIV/RSV) chimeras? Further, how does the Office's determination with regard to enablement of this subject matter in Applicants' specification relate to the Office's interpretation of the Belshe et al. disclosure (with the noted exception of PIV/measles chimeras)? Applicants respectfully request clarification as to the relevant factual criteria to be applied and considered in evaluating these questions. In this regard, Applicants note that MPEP § 2164.04 states that "the examiner should always look for enabled, allowable subject matter and communicate to applicant what that subject matter is at the earliest point possible in the prosecution of the application (emphasis in original)."

In clarifying the record pertaining to enablement of PIV chimeras, the Office is further urged to provide direct scientific evidence and reasoning for all of the relevant factors considered. In this context, Applicants' alleged "admission" relating to the level of predictability in the art does not qualify as evidence in the manner relied upon by the Office. In particular, this alleged admission was expressly limited to the predictability for achieving viable chimeric PIV vaccines before Applicants' invention. As stated by Applicants, the lack of predictability pertained only to the art as viewed before Applicants' filing date, "without specific data such as the results disclosed in Applicants' specification teaching, e.g., a viable PIV1-PIV3 chimera." It is therefore unfounded to interpret Applicants' remarks in this context

as an admission that the level of predictability in the art facing Applicants in the present case remains unpredictable. On the contrary, the record should clearly reflect that Applicants' expectations for success were justifiably buoyed by their own working examples proving successful construction of a number of viable chimeric PIV constructs within the claims, including viable HPIV3-1 chimeras and even more challenging chimeras incorporating attenuating mutations from JS *cp*45.

Patentability Under 35 U.S.C. §§ 102 and §103

Applicants acknowledge that the Office has reconsidered and withdrawn the rejection of claims 11, 48, 49, 52, 54, 57-59, 60, 91, and 93 under 35 U.S.C. 102(a) as allegedly anticipated by Kato et al (Genes to Cells 1:569-579, 1996) set forth in the previous Office Action.

Claims 1-4, 6, 7, 10-17, 20, 21, 26, 27, 30, 33-40, 43, 44, 47-49, 52, 54, 56, 57, 59, 61-85, 88-91, 93, 94, 96-116, 118, 120-143 stand rejected under 35 U.S.C. §102(e) as clearly anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over, Belshe et al (5,869,036). Applicants respectfully traverse.

The Examiner has kindly responded to Applicants' request for clarification regarding the nature and scope of the outstanding art rejection over Belshe et al. It now appears clear that the reference is interpreted by the Office to expressly anticipate at least the principal subject matter of the pending claims. Ancillary statements are provided by the Office (at pp. 6-8 of the Office Action, Paper No. 17) to clarify that certain other subject matter not expressly anticipated by the Belshe et al. claims (e.g., "an isolated infective virus") is "at least explicitly suggested by the patent claim" (e.g., "even if the claim is not squarely drawn to the virus per se"). On this basis, the Office Action is considered to maintain that the Belshe et al. disclosure anticipates the principal subject matter of the claims now presented for reconsideration.

Consistent with these clarified grounds of rejection, the present Office Action focuses on the issue of whether or not the Belshe et al. reference is enabling for the subject

matter of Applicants' invention (Office Action Paper No. 17, at pp. 8-9). In this context, the Office briefly considers Applicants' previous remarks assert that Belshe et al. does not enable the presently claimed subject matter. In reply to these remarks, the Office explains the nature of the outstanding rejection as follows:

> Applicant correctly states that the rejections are founded on the literal content of the claims set forth in the patent. The patented subject matter benefits from the legal presumption of validity, and therefore the subject matter of the claims is presumed enabled. (Office Action Paper No. 17, at pp. 5-6, underscores added).

Beyond this sweeping assertion that the claims of Belshe et al. are "presumed enabled", the Office provides only brief mention of Applicants' arguments submitted in rebuttal to this recognized presumption (see, Office Action Paper No. 17, at pp. 8-9). Whereas the Office makes a kind effort in an admittedly complex and detailed case to parse out individual aspects of the claimed subject matter and correlate this subject matter with the teachings of Belshe et al. (Office Action Paper No. 17, at pp. 5-7), the actual evidence presented by Applicants in their extensive prior response (see, June 19, 2000 Amendment, at pp. 8-22) on this subject is largely ignored. The treatment which the Office provides to this subject matter is limited to the following general dismissals and assertions.

At page 8 of the Office Action, the Examiner refers to Applicants' remarks characterizing the content of the Belshe specification as speculative, and based on incorrect predictions. In general response, the Office states that:

> [A] working example is not required for enablement, and applicant has not provided evidence that one skilled in the art would have been unable to practice the patented invention given the disclosure in the patent specification and the ordinary knowledge of those skilled in the art at the time the patent was filed.

Notably, this conclusion is made without referencing any of the specific items of evidence presented in Applicants' detailed, June 19, 2000 Amendment. In fact, the Office did not present any further detailed consideration, even though the evidence squarely relates to

factual issues that underlie the stated presumption of enablement for the Belshe et al. disclosure. Rather than consider this evidence, the Office did not address the issues raised in Applicants' Amendment and instead imposed an unrelated burden upon Applicants to show "critical" process steps or starting materials to distinguish the claims over the Belshe et al. disclosure. Thus, page 9 of the Office Action (Paper No. 17, emphasis supplied) states as follows:

> If, as applicant asserts, the teachings in Belshe are inadequate to enable the products and methods recited in the patent claims, can applicant point to a process step or starting material that is critical for the success of the invention, and point to where the critical feature is taught by applicant and not taught by Belshe et al? Applicant should be prepared to amend the claims to require that critical step or material, since (if it exists) the omission of the critical feature will amount to a gap between the method steps or a gap between the elements. See MPEP § 2172.01.

Applicants respectfully challenge this reallocation of the burden of proof on the issue of enablement, for the following reasons. First, Applicants have submitted extensive evidence to rebut the presumption that the Belshe et al. disclosure is enabling for the presently claimed subject matter. The Office has not evaluated this evidence in any kind of substantive detail that would provide Applicants an opportunity to respond to specific factual criteria deemed by the Office to support the rejection. Presently, the sole factual criterion advanced by the Office in this context is that "the rejections are founded on the literal content of the claims set forth in the patent." This perspective essentially ignores Applicants' rebuttal evidence. Secondly, the requirement for Applicants to identify "critical" process steps or starting materials to distinguish the invention from the Belshe et al. disclosure is believed improper. To support this requirement, the Office cites MPEP § 2172.01. However, this section of the Manual relates to "Unclaimed Essential Matter", which is clearly not the sole basis to support Applicants' rebuttal position on the issue of enablement. On the contrary, the factual inquiry for proper enablement review encompasses a broad range of factual determinations, as briefly discussed below.

For a patent to be valid, the disclosure must enable persons of ordinary skill in the art to practice the invention as broadly as it is claimed. 35 U.S.C. §112. "The sine qua non of a valid patent is a full, clear, *enabling* description of the invention." Ex parte DeCastro, 28 USPQ2d 1391, 1393 (BPAI 1993) (emphasis in original). Stated alternatively, the specification "must sufficiently describe the claimed invention to have placed the public in *possession* of it." In re Donohue, 226 USPQ 619, 621 (Fed. Cir. 1985) (emphasis supplied).

In practical terms, the enablement requirements of 35 U.S.C. §112 mean that the patent disclosure "must teach those skilled in the art how to make and use the full scope of the invention without 'undue experimentation.'" In re Wright, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). To determine whether undue experimentation is required, a variety of factors are considered, including:

(1) the quantity of experimentation necessary;
(2) the amount of direction and guidance provided by the specification;
(3) the presence or absence of working examples;
(4) the nature of the invention;
(5) the state of the prior art;
(6) the level of skill in the art;
(7) the predictability of the art; and
(8) the breadth of the claims.

In re Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988); Ex Parte Forman, 230 USPQ 546, 547 (BPAI 1986).

It is never proper for a patentee to rely upon knowledge or methods developed after the filing date of the application to supplement a disclosure. Gould v. Quigg, 3 USPQ2d 1302, 1305 (Fed. Cir. 1987); U.S. Steel Corp. v. Phillips Petroleum Co., 9 USPQ2d 1461, 1464 (Fed. Cir. 1989).

> The issue is not what the state of the art is today or what a skilled artisan today would believe, but rather what the state of the art was (at the time the application was filed) and what a skilled artisan would have believed at that time.

In re Wright, 27 USPQ2d 1510, 1514 (CPCF 1993). As further explained in In re Glass, 181 USPQ 31, 34 (CCPA 1974, emphasis supplied):

> It is an applicant's obligation to supply enabling disclosure without reliance on what others may publish after he has filed an application on what is supposed to be a completed invention and later issuing patents or publications may not be relied upon to establish that the specification is enabling under § 112, paragraph one.

See also, Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 13 USPQ2d 1737, 1794 (DC 1989):

> It is well-established that a state of the art coming into existence after the filing date of an application cannot be used in determining enablement under 35 U.S.C. § 112.

Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 13 USPQ2d 1737, 1794 (DC 1989).

An important contrast between Applicants' disclosure and that of Belshe et al. emphasizes the important role of "working examples" to support a generic patent claim in an unpredictable art. In the present case, the Office asserts that working examples are not required to meet the enablement requirements of 35 U.S.C. § 112 (Office Action Paper No. 17, at p. 9). However, the Forman and Wands decisions, *supra*, make it clear that working examples which demonstrate operability of a claimed invention are important factors to consider in determining enablement, particularly in unpredictable arts. This principle is embraced by the Patent Office and emphasized in the context of unpredictable inventions (MPEP § 2164.02), as follows:

> Lack of a working example, however, is a factor to be considered, especially in a case involving an unpredictable and undeveloped art (underscore added).

The requirement for working examples to support generic claims in chemical and molecular patents is well-founded. To be enabling, a patent disclosure must be accepted by the skilled artisan to actually place the public "in possession of" the invention. For broad claims reciting unpredictable subject matter, the absence of commensurate working examples, as in the case of Belshe et al., would generally be considered unreasonable from the standpoint of the skilled artisan. This rationale is reflected in the Board's decision in Ex parte Sudilovsky, 21 USPQ2d 1702, 1705 (BPAI 1991):

> When a patent applicant chooses to forego exemplification and bases utility on broad terminology and general allegations, he runs the risk that unless one with ordinary skill in the art would accept the allegations as obviously valid and correct, the examiner may, properly, ask for evidence to substantiate them.

Based on this same rationale, the patentee's failure to provide working examples to support generic molecular claims in a recent Federal Circuit case, Genentech Inc. v. Novo Nordisk A/S, 42 USPQ2d 1001, 1006 (1997), raised a strong inference against enablement:

> It stands to reason that if the disclosure of a useful conjugate protein and the method for its cleavage were so clearly within the skill of the art, it would have been expressly disclosed in the specification, and in the usual detail. Patent draftsmen are not loath to provide actual or constructive examples, with details, concerning how to make what they wish to claim. (emphasis supplied).

In setting forth the instant rejection, the Office expressly concedes that Belshe et al. "does not provide a working example of the material set forth in applicant's claims." (Office Action Paper No. 10, at p. 6). Instead, the rejections are founded on the literal content of the claims set forth in the Belshe et al. patent. To maintain the rejection, the record must be satisfied that the Belshe et al. disclosure fulfills all of the enablement requirements of 35 U.S.C. § 112.

> The standard for anticipation by patenting is the same one of a full enabling disclosure that applies to printed publications, i.e., it must disclose the invention in such full, clear and exact terms as to enable any person skilled in the art to which the invention relates to practice it.

Electronucleonics Laboratories, Inc. et al. v. Abbott Laboratories, 214 USPQ 139, 147 (N.D. Ill. 1981) (underscore added, citations omitted).

As further explained by the Federal Circuit in In re Donohue, 226 USPQ 619, (Fed. Cir. 1985).

> It is well settled that prior art under 35 U.S.C. § 102(b) must sufficiently describe the claimed invention to have placed the public in possession of it.
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> ***
>
> [E]ven if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it was not enabling. (emphasis supplied, citing In re Borst, 45 USPQ 544, 557 (CCPA 1965), *cert. den.* 382 U.S. 973, 148, USPQ 771 (1966).

In contrast to Applicants' disclosure which describes successful recovery of specific, attenuated, recombinant PIV vaccine candidates, the Belshe reference contains no "working example" of any such materials or methods as are set forth in Applicants' pending claims, which is expressly conceded by the Office (Office Action Paper No. 10, at p. 6). On the contrary, Belshe et al. describe the simple ability of a plasmid expressing a wild type PIV3 L protein to enhance replication of a biologically derived mutant PIV, JScp45, virus at a restrictive temperature of 39.5°C. These findings are offered as a basis for theorizing that the L gene of cp45 possesses mutations that might prove useful if they could be incorporated in a recombinant PIV vaccine virus derived from cDNA.

Belshe et al. described the potential utility of specific mutations in the cp45 strain. However, it must be recognized that the biologically derived mutant virus (cp45) recovered by Belshe et al., after complementation in the manner described above, was not changed or modified in any manner as provided by Applicants' disclosure. No cDNA constructs were designed and produced by Applicants, from which PIV3 wild type or mutant viruses could be recovered, and certainly no new constructs or recombinant viruses bearing specific, attenuating mutations were ever produced by Belshe et al. Resolving these limitations was critical to enablement of Applicants' claimed invention. At the same time, the absence of such disclosure in the Belshe et al. reference negates any "reasonable expectation for success" to achieve Applicants' invention. This is especially clear when the "particular results" achieved by Applicants are considered, namely that it was shown to be possible to construct recombinant PIV vaccine candidates from cDNA that are suitably attenuated, yet sufficiently immunogenic to produce a protective immune response in immunized hosts.

In order to determine whether such a recombinant virus contains the complex set of biological properties necessary for development of a live attenuated virus vaccine (including viability, attenuation, immunogenetic, and protective efficacy), it is first necessary to generate recombinant viruses using cDNA technology as disclosed by Applicants. The present application provides the first description of recovery of infectious PIV from cDNA, as well as recovery of specific, attenuated PIV vaccine candidates as described in the specification.. Only after successfully developing and implementing this recovery system can it be subsequently shown that the phenotypic effect of any desired mutation, e.g., from a biologically derived cp45 mutant (for example a ts mutation identified in L), can be segregated from complementary or interactive effects of other mutations.

With regard to Applicants' basic cDNA recovery method, it is important that the Office consider important deficiencies in the art at the time of the invention relating to unique aspects of PIV recovery systems. In particular, the PIV rescue system disclosed by Applicants departs in fundamental and significant aspects from other rescue systems for negative stranded RNA viruses, including for example rabies and VSV rescue systems. At the same time, the human PIV3 rescue system disclosed by Applicants differs substantially from the murine PIV (Sendai) virus recovery system reported by Kato et al. (of record). For example, an important aspect of Applicants' PIV3 rescue system that is not disclosed or suggested by the cited references is the distinct organization of the P gene. The P gene of human PIV3 differs from that of all other systems described in the art of record. In particular, the HPIV3 P gene contains ORFs that can encode four distinct proteins, P, C, D, and V. In contrast, VSV and rabies only encode one P-related protein. Sendai virus encodes a P protein, several C proteins that are carboxy co-terminal, and a V protein. None of these other viruses contain an ORF related to the PIV3 D protein. Since prior rescue systems required the expression of L, N and P proteins, it was unknown at the time of the invention whether successful HPIV3 recovery would require expression of the D ORF (as disclosed by Applicants in construction of the pTM(P) expression plasmid).

Concerning more detailed aspects of the invention, it is Applicants' position that the disclosure of Belshe et al. does not enable production, in a cDNA-based recovery system,

of an infectious PIV that is suitably attenuated by incorporation of one or more recombinantly introduced mutation(s), or by other methods disclosed in Applicants' specification, nor does the Belshe et al. reference enable yet more challenging aspects of Applicants' invention, such as production of infectious, chimeric PIV for use in vaccine formulations. The simple complementation assays described by Belshe et al. using the biologically derived mutant cp45 virus and a wild type L plasmid, was only conducted *in vitro* using tissue culture cells, and was not validated by parallel studies *in vivo*. From this disclosure, the report that replication of cp45 can be complemented by wild type L protein in tissue culture cells does not amount to a scientific forecast that a recombinant virus bearing one or more of the cp45 mutations would be attenuated *in vivo*. This correlative deficiency is apparent from the following considerations.

As previously stated in the record, it was well known at the time of the invention that "temperature-dependent hose range (td-hr) mutant" viruses of various groups may be temperature sensitive (ts) on one tissue culture cell but not on others. Furthermore, these td-hr mutants do not necessarily exhibit their *in vitro* attenuated phenotype *in vivo* (see Snyder et al., Virus Research 15:69-84, 1990 and Shimizu et al., Virology 124:35-44, 1983, of record). As described in Snyder et al., an exemplary mutant (clone 143-1) of influenza virus was shown to be highly ts in tissue culture cells, but was not significantly attenuated *in vivo*. Additional findings by Shimizu et al. indicate that such phenotype differences among td-hr mutants are common and are found in many different complementation groups of the influenza virus (i.e., they are present in many different genes of the virus).

The Belshe et al. reference does not demonstrate whether any of the contemplated ts mutations in the L gene of cp45 belong in the td-hr class of mutations, or in the other class of ts mutations whose replication is effected by the temperature present in the host animal. In view of this deficiency, the simple description of a complementation phenotype for a group of multiple, unsegregated mutations in a complete gene *in vitro* does not serve as a reliable indicator of attenuation *in vivo*.

In addition to this deficiency, the Belshe et al. reference does not describe specific levels of temperature sensitivity and/or attenuation for any virus bearing one or more of the three identified cp45 L gene mutations. This important property for assessing vaccine strains cannot be reliably inferred from the *in vitro* complementation system employed by Belshe et al. to enable a recombinant, live-attenuated PIV vaccine candidate. On the contrary, the teachings of Belshe et al. merely show that the cp45 mutations in L make some contribution to a ts phenotype *in vitro* in a particular cell line. From these limited teachings, it cannot be reasonably predicted what level of temperature sensitivity and/or attenuation a recombinant virus that might be engineered to incorporate one or more of the L gene mutations of cp45 would exhibit. For example, if the contribution of the set of cp45 L mutations to the overall level of temperature sensitivity of cp45 was small, and a virus bearing all three cp45 mutations in L was restricted at 39.5°C (the only temperature tested by Belshe et al.) but not at 37°C, such a virus may not be attenuated at all in a host with a 37°C body temperature. Thus, the disclosure of Belshe et al. does not reasonably demonstrate that incorporation of the cp45 L gene mutations would yield a recombinant virus with useful properties for vaccine use, which could only have been determined using an actual live, infectious viral recovery system as disclosed by Applicants.

The complex interactions and effects of mutations in the PIV3 cp45 virus that determine its level of temperature sensitivity and attenuation, demonstrated in the present application, clearly show that the *in vivo* properties of individual and collective mutations in recombinant PIVs cannot be reliably predicted from *in vitro* complementation studies as presented by Belshe et al. For example, prior to Applicants' invention it was not predictable that the temperature sensitive phenotypes of the cp45 L gene mutations recovered in recombinant PIVs engineered from cDNA would not be not additive. However, as revealed in the instant specification, the assembly of two cp45 (992 and 1558) mutations yielded a recombinant virus bearing multiple ts mutations that was less temperature sensitive compared to recombinants bearing either single mutant. It was only through the implementation of Applicants' technology that these unexpected results were discerned and recognized to provide

useful tools for calibrating attenuation and immunogenicity in recombinant PIV vaccine candidates.

In relation to the foregoing points, the Belshe reference is cited by the Office as teaching that a temperature sensitive phenotype accurately predicts the presence of an attenuation phenotype in a recombinant PIV. Applicants' disclosure reveals the flawed nature of this conclusion. A recombinant HPIV3 or a recombinant hybrid virus bearing one or more of the cp45 mutations in L was never recovered by Belshe. Since a recombinant virus bearing a cp45 L mutation was not recovered by Belshe et al., the authors could not demonstrate that the mutations in the L gene of cp45 are determinants of attenuation since this can only be determined by the demonstration that the mutation confers the attenuation phenotype when introduced into a wild type recombinant virus. Belshe and coworkers also did not demonstrate that one or more of the mutations in L can specify the attenuation phenotype independent of the other cp45 mutations. This was not accomplished, because the disclosure did not teach how to recover a recombinant virus, either a HPIV3 virus or one of the presently described chimeric viruses.

In contrast to the prophetic teachings of Belshe et al., Applicants' specification teaches that the rcp45 3'N recombinant was ts but not attenuated and, conversely, the rcp45 C and rcp45 F recombinants were attenuated and not ts. Furthermore, r942/992, which exhibited a level of temperature sensitivity comparable to that of cp45 virus, was overattenuated *in vivo*. Conversely, r992/1558 was much less attenuated that cp45. These unexpected findings underscore the deficiencies of the Belshe et al. patent— which does not identify specific properties determined by individual cp45 L gene mutations, much less to reliably predict combinatorial phenotypes specified by sets of mutations incorporated within novel recombinant vaccine candidates and analyzed *in vivo*. Only through the use of Applicants' successful cDNA recovery system could these unexpected effects be determined and harnessed for use within the claimed methods and compositions.

In further detailed aspects of the instant disclosure, five attenuating mutations, are identified and directly characterized in the cp45 mutant (in C, F, and L), three of which

were shown to specify ts mutations, and two which were non-ts attenuating mutations. Through the use of Applicants' recovery system, the ability of these mutations to independently confer the property of attenuation on a recombinant virus in the absence of other cp45 mutations indicated their general usefulness for attenuating recombinant, including chimeric recombinant, vaccine viruses. Such findings were not supported by the disclosure of Belshe et al. On the contrary, the mutation characterized in the C coding sequence was not even identified in the Belshe patent. This is a non-ts attenuating mutation in the C protein of cp45.

Also in the present disclosure, Applicants demonstrated that recombinant virus bearing the 3' leader and N gene mutations (rcp45 3'N in Tables 9 and 11) was ts, but was not attenuated *in vivo*. Belshe et al. did not recognize that the cp45 mutations in N or the 3' leader region contributed to the ts phenotype. This is because Belshe et al. provided an incorrect N gene sequence, and thus clearly did not provide a written description of any recombinant virus having such a recombinant member. This underscores the limitations of the Belshe et al. disclosure, particularly relating to the lack of a working example of a cDNA recovery system for PIV, discussed above. Briefly, as indicated in Table 3 of the Belshe et al. reference, the combination of L, P, and N plasmids expressing wild type HPIV3 proteins reportedly increased the level of replication cp45 about 10-fold over that of the combination of P and L alone. From this data, Belshe et al. might have also proposed that the cp45 N gene is a ts gene (i.e., that the N gene product, like the L protein, complemented growth of cp45 in conjunction with L). However, this hypothesis was not advanced by Belshe et al., which likely relates to a sequence error in the Belshe et al. disclosure relating to the sequence for cp45 N. As indicated in Figure 1 of Belshe et al., neither the N protein nor the non-coding regions of the N gene, contain mutations. However, as indicated in Table 8 of the present application, there are in fact two coding mutations in the N gene of cp45, as well as an additional mutation in the gene start sequence of the N gene. Thus, the cp45 sequence of the N gene in Figure 1 of the Belshe et al. disclosure lacks three confirmed mutations in cp45.

If Belshe et al. had provided the correct sequence of the N gene, the reasoning applied by the Office would necessarily suggest that these mutations, like those discussed for

the L gene, would be useful in designing a recombinant vaccine virus. However, the data presented in Applicants' working examples (see, e.g., Tables 9 and 11 of the specification) indicate that these ts mutations do not specify an attenuation phenotype, and therefore would not be useful in a vaccine virus. This discrepancy between hypothetical and working descriptions underscores the basic assertions of Applicants', which hold that (1) the *in vitro* property of temperature sensitivity does not reliably predict attenuation *in vivo* and, (2) that useful mutations for incorporation in Applicants' recombinant vaccine viruses can only be defined by actual recovery of the mutations in a recombinant virus, and by demonstration that the mutations specify desired phenotypes in the recombinant virus.

In view of the foregoing evidence, it is respectfully submitted that the foundational cDNA recovery system for PIV, first developed by Applicants, is neither taught nor suggested by the disclosure of Belshe et al., alone or in any combination with the remaining art of record. Concerning more detailed aspects of the invention, the disclosure of Belshe et al. clearly does not enable production, in a cDNA-based recovery system, of infectious PIV that are suitably attenuated by incorporation of one or more recombinantly introduced mutation or by other methods described by Applicants.

Claims 51 and 53 are rejected under 35 U.S.C. §103(a) as being unpatentable over Belshe et al, for reasons of record. The Office Applicant argues specifically that "including all of the required nucleic acid sequences in a single vector would have been obvious." In response, Applicants respectfully submit that the basic cDNA recovery method, yielding "an infectious PIV particle" of claim 48 is patentable over Belshe et al. for the reasons noted above. Claims 51 and 53 depend directly or indirectly from claim 48 and necessarily include all elements and limitations therein. Therefore, claims 51 and 53 are also patentable over Belshe et al., irrespective of the merits of the Office's discussion regarding single versus multiple vector expression systems, which need not be addressed here.

Claims 18, 19, 28 and 29 are rejected under 35 U.S.C. §102(e) as anticipated by Belshe at al., or, in the alternative, under 35 U.S.C. §103(a) as obvious over Belshe et al. in view of Stokes et al. (Virus Research 30:43-52, 1993). Applicants respectfully traverse. In

this regard it is submitted that the teachings of Belshe et al. do not render the subject matter of the rejected claims anticipated or obvious, for the reasons noted above. Further, there is no additional disclosure in the Belshe et al. or Stokes et al. references that would render recombinant RSV having specific substitutions in the N protein (see above) enabled or provide a reasonable expectation of success for achieving such recombinants having the characteristics disclosed by Applicants.

Claims 22-25, 31, 32, 42, 60, 117, and 119 are rejected under 35 U.S.C. §103(a) as being unpatentable over Belshe et al. in view of Conzelman (J. Gen. Virol. 77:381-389, 1996). The teachings of Belshe et al. do not render the subject matter of the rejected claims anticipated or obvious, for the reasons noted above. The general review by Conzelman does not add to the disclosure of Belshe et al. so as to enable the subject claims or provide a reasonable expectation of success for achieving the claimed subject matter and results disclosed by Applicants.

Claims 11, 48, 50, 52, 55, 56, 58, 91 and 92 are rejected under 35 U.S.C. §102(b) as being anticipated by Dimock et al. (J. Virol. 67:2772-2778, 1993), for reasons of record. The Office acknowledges Applicants previous comments relating to the limited disclosure in Dimock et al. of a minigenome system. However, the Office urges Applicants to clarify "[h]ow is the minigenome of the reference different from the PIV genome modified by deletion as recited in claim 11?", and "[h] ow is the defective PIV of the reference different from a recombinant "subviral particles" of claims 91-92." An additional issue raised by the Office relates to the interpretation of the term "expression vector". However, it is not clear how the use of this term is considered "confusing" by the Office, and clarification or suggestion of proposed amendatory language is thus respectfully requested.

Applicants maintain traverse against the foregoing grounds of rejection and submit that the subject matter of claims 11, 48, 50, 52, 55, 56, 58, 91 and 92 is neither disclosed nor suggested by Dimock et al. As noted previously in the record, the Dimock et al. reference discloses a limited, minigenome system for recovering synthetic "analogs" of genomic RNA and replicative-intermediate RNA of HPIV3. The reference clearly does not

disclose the polynucleotide of claim 11 encoding a PIV genome or antigenome as recited (now amended to further recite that "said polynucleotide upon coexpression with PIV N, P and L proteins yields an infectious PIV particle"). In this latter context, it is quite clear that the synthetic analogs described by Dimock et al. and others, including "minireplicons", incorporate only a small fraction of the total viral genome or antigenome sequence, and may not include even a single intact viral gene therein. Minireplicons contrast starkly with a full length antigenome as incorporated in Applicant's rescue system, which comprises a full complement of viral genes that were shown to be fully functional by actual recovery of infectious, self-replicating viral particles. Reports concerning replication of short "minireplicons" have little predictive bearing on the replication of a more complete PIV genome or antigenome containing multiple genes. Moreover, these systems provide no evidence for the formation of extracellular particles of any kind, much less of extracellular particles that direct transcription or are self-replicating.

Thus, there is no guidance as to which viral proteins must be included to provide for such particles, nor how functional cDNAs for such proteins might be identified, nor how such particles might be isolated from the transfection harvest, which would include a very large background of vaccinia virus. In the absence of demonstrated transcription and particle formation, two critical and complex aspects of any PIV recovery system, minireplicon and complementation recovery systems provide insufficient direction and guidance for the artisan to achieve even the more fundamental aspects of the presently claimed subject matter, without undue experimentation.

Accordingly, the "defective particles" of Dimock et al. do not represent "an infectious PIV particle", and this or comparable language is now within all of the claims to which the rejection is applied. With respect to the compositions of claims 48 and 50, the methods of claims 52, 55, 56, and 58 or the infectious PIV particles of claims 91 and 92, these all involve admixture, coexpression or association of a PIV genome or antigenome and N, P, and L proteins to produce an infectious PIV particle. Dimock et al. fails to anticipate admixture, coexpression or association of a PIV genome or antigenome and N, P, and L proteins to produce an infectious PIV particle. Thus, the rejection of claims 11, 48, 50, 52, 55,

56, 58, 91 and 92 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Dimock et al. is believed to be overcome.

Double Patenting

Claims 1-94 and 96-143 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6, 8-45, 47-50 of copending Application No. 09/458,813. The Office states that, although the conflicting claims are not identical, they are not patentably distinct from each other because they overlap in scope with the instant claims.

Claims 1-94 and 96-143 are also provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-58 of copending Application No. 09/459,062. The Office states that, although the conflicting claims are not identical, they are not patentably distinct from each other because they overlap in scope with the instant claims.

Applicants acknowledge the provisional obviousness-type double patenting rejections, and respectfully decline to address the merits of the rejections until subject claims in one of the identified applications is allowed.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: April 5, 2001

Jeffrey J. King
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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 27, line 12, has been amended as follows:

--In one aspect of the invention, mutations occurring in biologically derived, attenuated PIV are identified and introduced individually or in combination into a full-length PIV clone, and the phenotypes of rescued recombinant viruses containing the introduced mutations are determined. In exemplary embodiments, amino acid changes displayed by biologically derived mutant viruses over a wild-type PIV, for example changes exhibited by PIV mutants having ts, ca or att phenotypes, are incorporated within recombinant PIV clones. These changes from biologically derived mutant PIV specify desired characteristics in the resultant clones, e.g., an attenuation phenotype specified by a mutation adopted from the HPIV3 mutant JS cp45 deposited in accordance with the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Blvd. Manassas, VA 20110-2209, U.S.A., and granted the designation PTA-2419. These changes are preferably introduced into recombinant virus using two or three nucleotide changes compared to a corresponding wild type or biologically derived mutant sequence, which has the effect of stabilizing the mutation against genetic reversion.--

IN THE CLAIMS:

Claims 11, 39, 55, 131, 132, 134, 136, 139-141, and 143 have been amended as follows:

11. (Twice Amended) An isolated polynucleotide molecule comprising an operably linked transcriptional promoter, a polynucleotide sequence encoding a human or bovine PIV genome or antigenome, and a transcriptional terminator, wherein said polynucleotide sequence encoding said PIV genome or antigenome is modified by a nucleotide

insertion, rearrangement, deletion or substitution, whereby said polynucleotide upon coexpression with PIV N, P and L proteins yields an infectious PIV particle.

39. (Twice Amended) The isolated polynucleotide molecule of claim 33, wherein said chimeric genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present in rcp45, rcp45 3'NCMFHN, rcp45 3'NL, rcp45 3'N, or rcp45 F other than mutations in HN and F, selected from i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

55. (Amended) The method of claim 52, wherein [at least one of] the N, P and L proteins [is supplied by coinfection with PIV] are encoded on three different expression vectors.

131. (Amended) The isolated polynucleotide molecule of claim 130, wherein said one or more mutations of JS cp45 comprise a plurality and up to a full complement of mutations present in JS cp45 other than mutations in HN and F, selected from i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

132. (Three Times Amended) The isolated polynucleotide molecule of claim 129, wherein the isolated polynucleotide encoding the chimeric PIV genome or antigenome further incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of [Thre] Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

134. (Three Times Amended) The isolated polynucleotide molecule of claim 133, wherein said chimeric genome or antigenome incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of [Thre] Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

136. (Three Times Amended) The method of claim 135, wherein said genome or antigenome incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of [Thre] Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28,

and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

139. (Amended) The isolated infectious PIV particle of claim 138, wherein said one or more mutations of JS cp45 comprise a plurality and up to a full complement of mutations present in JS cp45 other than mutations in HN and F, selected from i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

140. (Three Times Amended) The isolated infectious PIV particle of claim 137, wherein the isolated polynucleotide encoding the chimeric PIV genome or antigenome further incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of [Thre] Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

141. (Three Times Amended) The isolated infectious PIV particle of claim 111, wherein said chimeric PIV genome or antigenome further incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of

[Thre] Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.

143. (Three Times Amended) The immunogenic composition of claim 142, wherein said recombinant PIV genome or antigenome further incorporates mutations comprising i) substitutions specifying a replacement of His for Tyr942, Phe for Leu992, and Ile for Thr1558 in the polymerase L protein; ii) substitutions specifying a replacement of Ala for Val96 and Ala for Ser389 in the N protein; iii) a substitution specifying a replacement of [Thre] Thr for Ile96 in the C protein iv) mutations in a 3' leader sequence comprising a T to C change at a position corresponding to nucleotide 23 of JS cp45, a C to T change at nucleotide 24, a G to T change at nucleotide 28, and a T to A change at nucleotide 45 of JS cp45; and v) a mutation in an N gene start sequence comprising an A to T change at a position corresponding to nucleotide 62 of JS cp45.